REMARKS

Claims 3 to 9 and 11 as set forth in Appendix II of this paper are now pending in this case. Claims 1, 2 and 10 have been canceled, Claims 3 to 9 have been amended, and Claim 11 has been added, as indicated in the Listing of Claims set forth in Appendix I of this paper.

Applicants have amended Claim 3 to relate to a method of using the chroman based on the wording of Claim 9 in conjunction with the disclosure on page 2, indicated lines 21 to 36, of the application. Claims 4 to 8 have been revised accordingly. Claim 9 has been amended to relate to the subject matter of Claim 10. New Claim 11 has been added which specifies the concentration of the preparation defined in Claim 9 as set forth in Claim 8 with regard to the method. No new matter has been added.

The Examiner has rejected Claims 1 to 8 under Sections 101 and 112, ¶2, for being drawn to a "use" without reciting the requisite process step. Applicants have canceled Claims 1 and 2, and have amended the Claims 3 to 8 to relate to a method which requires the application of the chroman Ia in effective amounts. Withdrawal of the Examiner's rejection is, therefore, respectfully solicited.

The Examiner has rejected Claims 9 and 10 under 35 U.S.C. §103(a) as being unpatentable in light of the disclosure of **Jiang et al.** (CA 134:759916, (2000) which reports an article published in PNAS 97(21), 11494-11499 (2000)¹⁾). The respective publication relates to the inhibition of cyclooxygenase activity in macrophages and in epithelial cells with γ -tocopherol and with its major metabolite γ -CEHC. The authors report that their investigations with α -tocopherol showed no effect on epithelial cells. Investigations with α -CEHC are not reported²).

Applicants' formula Ia represents $\alpha\text{-CEHC}^3$. Applicants have found that the application of effective amounts of $\alpha\text{-CEHC}$ protect the human skin and the human hair against aging processes and harmful environmental effects. The Examiner will note that $\alpha\text{-tocopherol}$ differs

¹⁾ A copy of Jiang et al.'s article is herewith enclosed for the Examiner's convenience.

²⁾ For the structural formulae of α -tocopherol, α -CEHC, γ -tocopherol and γ -CEHC see the attached Appendix III.

³⁾ Note, for example, page 4, indicated line 10 et seq., of the application.

structurally from γ -tocopherol in the presence of a methyl group in 5-position of the chroman ring.

Jiang tal.'s teaching that α -tocopherol, in contrast to γ -tocopherol, had no effect on epithelial cells shows that it is not possible in this particular area to assume that a homolog, ie. a compounds which merely differs in one CH₂ moiety, will have similar properties. Accordingly, a person of ordinary skill in the pertinent art cannot reasonably expect that the effect on epithelial cells which is found when γ -CEHC is employed will equally be found when a compound which structurally differs from γ -CEHC due to a CH₃ group in 5-position of the chroman ring, namely α -CEHC, is used. Based on the teaching of Jiang et al., a person of ordinary skill in the pertinent art could therefore not reasonably expect to find any beneficial effects when α -CEHC is applied to the skin or the hair.

It is well established that a compound and its properties are inseparable⁴⁾, and that the inventive subject matter as a whole, which is referred to in the statute, embraces not only the subject matter particularly recited in the claims, but also the properties which are inherent in the particular combination of features defined in the claims, as well as the problem which is solved⁵⁾.

Favorable reconsideration of the Examiner's position and with-drawal of the rejection under Section 103(a) is therefore respectfully solicited.

The method which is defined in Claims 3 and further specified in Claims 4 to 8 relates to the utilization of α -CEHC for protecting the skin and the hair, and the reasons set forth with regard to Claims 9 and 11 are equally applicable where the obviousness of Claims 3 to 8 in light of the teaching of *Jiang et al.* is concerned.

In light of the foregoing and the attached, the application should be in condition for allowance. Early action is respectfully solicited.

⁴⁾ ie. <u>In re Papesch</u>, 315 F.2d 281, 137 USPQ 43 (CCPA 1963)

⁵⁾ ie. <u>In re Antonie</u>, 559 F.2d 618, 195 USPQ 6 (CCPA 1977); <u>In re Wright</u>, 848 F.2d 1216, 6 USPQ2d 1959 (Fed. Cir. 1988), overruled on other grounds in <u>In re Dillon</u>, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1990) (en banc), cert. denied 500 U.S. 904 (1991)

REQUEST FOR EXTENSION OF TIME:

It is respectfully requested that a one month extension of time be granted in this case. A check for the \$110.00 fee is attached.

Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees, to Deposit Account No. 11.0345. Please credit any excess fees to such deposit account.

Respectfully submitted, KEIL & WEINKAUF

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Encl.: THE LISTING OF CLAIMS (Appendix I)

THE LISTING OF CLAIMS (Appendix II)

STRUCTURAL FORMULAE (Sppendix III)

Jiang et al., NPAS 97(21), 11494-11499 (200)

HBK/BAS

APPENDIX I:

THE LISTING OF CLAIMS (version with markings):

- 1. (canceled)
- 2. (canceled)
- 3. (currently amended) [The use of] A method for protecting human skin or human hair against aging processes or harmful environmental effects which comprises applying to the skin or the hair an effective amount of a chroman [derivative as claimed in claim 1 having the] of formula Ia

$$\begin{array}{c} \text{CH}_3 \\ \text{H}_3\text{C} \\ \text{CH}_3 \\ \text{CH}_3 \end{array} \qquad \text{COOH}$$

- 4. (currently amended) The [use as claimed in claim 1] method of claim 3, wherein the chroman is applied for prophylaxis against aging processes of the human skin.
- 5. (currently amended) The [use as claimed in] method of claim 4, wherein the chroman is applied for prophylaxis against dry skin, wrinkle formation and/or pigment disorders.
- 6. (currently amended) The [use as claimed in claim 1] method of claim 3, wherein the chroman is applied for prophylaxis against aging processes of human hair.
- 7. (currently amended) The [use as claimed in] method of claim [1] 3, wherein [at least one of the compounds of the formula I is present in an] the effective [content in] amount of the chroman is applied by way of applying a cosmetic [preparations] preparation.
- 8. (currently amended) The [use as claimed in] method of claim 7, wherein [at least one of the compounds of the formula I is present] the cosmetic preparation contains the chroman in concentrations of from 0.01 to 30% by weight, based on the total amount of the cosmetic preparation.
- 9. (currently amended) A cosmetic preparation for protecting the human epidermis or human hair, which comprises, in a cosmetically suit-

able carrier, a cosmetically effective amount of [at least one of the compounds] a chroman of [the] formula Ia

$$R^{2}O$$
 R^{3}
 $COOR^{5}$
 CH_{3}
 $COOH$
 CH_{3}
 $COOH$
 CH_{3}
 $COOH$

 $[\frac{in-which-the-substituents-R^1-to-R^5-have-the-meanings-defined-according to-claim-1.}]$

- 10. (canceled)
- 11. (new) The cosmetic preparation defined in claim 9, which comprises the chroman in an amount of from 0.01 to 30% by weight, based on the total weight of the preparation.

APPENDIX II:

THE AMENDED CLAIMS (clean version):

- 1. (canceled)
- 2. (canceled)
- 3. (currently amended) A method for protecting human skin or human hair against aging processes or harmful environmental effects which comprises applying to the skin or the hair an effective amount of a chroman of formula Ia

$$H_3$$
C CH_3 $COOH$ CH_3

- 4. (currently amended) The method of claim 3, wherein the chroman is applied for prophylaxis against aging processes of the human skin.
- 5. (currently amended) The method of claim 4, wherein the chroman is applied for prophylaxis against dry skin, wrinkle formation and/or pigment disorders.
- 6. (currently amended) The method of claim 3, wherein the chroman is applied for prophylaxis against aging processes of human hair.
- 7. (currently amended) The method of claim 3, wherein the effective amount of the chroman is applied by way of applying a cosmetic preparation.
- 8. (currently amended) The method of claim 7, wherein the cosmetic preparation contains the chroman in concentrations of from 0.01 to 30% by weight, based on the total amount of the cosmetic preparation.
- 9. (currently amended) A cosmetic preparation for protecting the human epidermis or human hair, which comprises, in a cosmetically suitable carrier, a cosmetically effective amount of a chroman of formula Ia

$$\begin{array}{c} \text{CH}_3 \\ \text{H}_3\text{C} \\ \\ \text{CH}_3 \\ \\ \text{CH}_3 \\ \end{array} \\ \text{COOH} \\ \\ \text{Ia.}$$

10. (canceled)

11. (new) The cosmetic preparation defined in claim 9, which comprises the chroman in an amount of from 0.01 to 30% by weight, based on the total weight of the preparation.

APPENDIX III:

STRUCTURAL FORMULAE:

γ -Tocopherol and its major metabolite, in contrast to lpha-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells

Qing Jiang, Ilan Eison-Schweb. Chamal Courtemantho, and Bruce N. Ames*

Olabon of Bechmisty and Makemilas Biology, Unhandry of Cordenils, Barkalay, CA 907.87; and Chidaless Hoopial Oldand Research Institute, 5100 M. L. King II. Way, Olabund, CA gayos

Contributed by Buce H. Amer. 1019 78, 2000

that prizophend (17) reduced PCE, synchester the bit populated by the prizophend (17) reduced PCE, synchester the bit populated by the prizophend (18) the prizophend by the prizophend by the prizophend (18) In AA contempration, supporting that they might compete with AA or of white accuration, supporting that the observed a moderate and with the or of industrial manners of industrial manners or the opportunities of the or of production of the or of production of the opportunities of the or of the o Odoungmase-2 (COX-1)-cubrond ynthesis of protestation for PKES) plays a tey role in Inflammaton and Its associated diseases, such as uncorandorated by heart disease, those was report historion of CDX.3 activity, rather than affecting protein expression or unbarbase as waitability, and appeared to be inche paradom of autolotalem activity. PCRTC also inhibited POEs synthesis when exposed for 1 h is COX.2 periodic ed cells followed by the addition of arabidosic add (AQ), whereas under similar conflicion, yt required on 8- to 244 incutorion period to 4200 de énhitation. The allabitory potency of yt and YCRIC was diminished by an increase

Adhamatory diverses affect millions of peculie in the world, a short frome intramentation is one of the mijor contribution to acade development of careve as well a methodocorrelies and ordineascular diseases (1, 2). Aniouslant visualism which a confirm are believed to play an important rotation, which are formed to play an important rotation in fluxmoner of the methodocorrelies of the play an important rotation in the many court and the establishes organization (1). Among these visitables, as locacide or of (27), the predominant form of visitable band the establishes computent in one or vitamin is to many disturbed as returney a tradited both, in your and is vive (3, 4). In one is in V. dieu (5), was morely looved in the past because 1), which result in a post blocal single or the result in a post blocalisty as defined by the rest fees of buttons.

However, emerging evidence indicates that IT may be impor-tant to the determ against degenerative diseases. Some equico principal and incaparithat high intakes or high places kevil-of of predict from incaparite of beard disease (8, 10), whereast others fall to deserve the inverse correlation (11, 12) or pre-ference effects from all inverse correlation (11, 12). Instead, in the insulance of consury heart diseases. Cornery or al. (16) and our laboratory (17) have shown that y't is superior to all in soveral independent inventigations (11, 12, 15) demonstrate that plants concentrate that plants of yF, but not off, are inversely correlated Orgonog reactive altogen axide species (NOX), mulageale elec-proplika generated during inflammation. Recause of the cog-

intentional 5 posibon, 7T is a better muckeopalle and detroxided NCA by forming a studies advert, 5-nim or 7f (17-19), in min into pattern for comparing a new for catablists trouger inhibition of lipid personatasio induced by permynibite (17). Divisity 7T is primarily necessary to 2.78 trimeshyl-2 (p-carbonychir); by this systeman (FEHC), a water soluble compound found We have received because that y's implement of the library of the catabonychir); but human urine said peacesaing mediures activity (20, 21).

the lathibition of protein nitration and associate condition, and spaces vitantic C in its with spracationduced peritonitis (0.), 1. Lyterokidi, E. T. Shigero, B.N.A., M. K. Shigeroga, and S. Noc., il sprata bad yil plays a role to defending against inflammation related damage. In the present inflat, we divestigated the effects of yil on the inflammatory imposed with the control of the present inflam, we divestigated the effects of yil on the inflammatory impose in once iopheges and human epithehia colis. We found that IT thibblied the generation of prostaglandia Bi (PCEs), an important andiatry sputhesized via the cyclioatysenses 2 (OOX-2)-catshy and reads about 10 statement of AAA) dering and fammation. On reads about 10 to 17 statement of AAA) dering and fammation. On PAEDE, a physiological concentration are effective in inthe line.

Materials and Methods

MATIONALM, OT (1993) and yT (195-9753) were purchased from Acres Organics (Survoverville, NJ) or Flaka, y-CBHC (2-98%) and bight hydroperonds-free AA were from Gyman Gemicalle, Andlewylar, Prober, Trout-onlinersecon (DCH-H) was from GIBGO/BRL, Borterial lipopohysuchanite (LPS, B. Codiffs, Borterial lipopohysuchanite (LPS, B. Codiffs, Bart from Diffe, IL-/B and all other etermicals were from GISSB) was from Diffe, IL-/B and all other etermicals were from Signar.

GU Quhan. Murine RAV7264.7 metapohapet were murinely cultured in DMFM consulaing 10% FBS Human epithetist and cells (ASA9) were obtained from American The Qulture Collection and cultured in F12K medium apprecented with 10% FBS.

Cellulas Opule of at and 11. Cells were incubated in Daffer, containing 0.5% FBS supplemented with 10 pah of or 11 for 14 h. After harvested by scraping, cells were washed wribe with

Albertonne AA, erzelden leiző, Citá, rickustysmagi Bőfel Zí, i Zírákzol leimen ARCK helsző mel csete at tember (EA ligea Mandab kitá költ költésse richapin es él Arck at Kis, piszaj jedő fig felős rickaz szonyalkától bő, el, elseszékrek kil, piszajá-ent yeltet, ji Járálmogá i Egészőkkét kirjángotásonas.

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Healer balanced sell solution, of rad yT ware integrated by IPPLC by bying electrodybefueld aftercape, The amounts of off and \foatilde{T} in measurphages were 0.87 ± 0.12 and 1.03 ± 0.1 and \foatilde{T} in measurphages were 0.87 ± 0.12 and 1.03 ± 0.1 and \foatilde{T} in \foatilde{T} i 0.2 nmol/10° cells, respectively. PGS Generation themse Orients UPs and B. 19 for transful. R AVZ64.7 R (2016) for a vell) were allowed to entach overagit in a p 24-well piece Cardiluter (extlement candibated with DMRM a coolaining 0.5% FIS write entante (control) or (coopherol) for the lodicated time and then 0.1 µg/ml UPS was tempoherol for it is indicated time and then 0.1 µg/ml UPS was tempoherol for it is indicated with L DMRM containing 0.5% FIS supplemented with L DMRM containing 0.5% FIS supplemented with 10 pMRM containing 0.5% FIS supplemented with 10 pdf ml LLI for 24 h, at whith time the mertium was collected and PGE, p generabon was meanwed.

CORT ARTHMY to Inteat Cille. AX9 cells were presented with 10 byland. It is for X h, then incubited with free medium constituting tecophesols or elbands for the indicated fare, and foully icochaised with erogenous AA for 10 aim. PGE; release was measured as no indea of COX-2 activity.

determined by ottrie recommulation in LPS-activated many-physics by vough the Cross seasy (22). The generation of total reactive orngen species was evaluated by the oristates confluoratean DCFH to the highly Moneascent 2.7-dictalor/fluorascent, as monollosed by the increase in fluora-core idensity 1320 con with evelusion at 443 nm (23) by uning a Crioflinor 2350 fluorescent measurement system (Müllipsee). Withtle Measurement and DOM Oxidation. Formation of NOx was

ispirations synthesis were determined by an emyraulic lumu-nonssay (Cayman Chemicals), in which highly speaks makes were used POD, was far converted to POD, methodine by resting with realborytamine and the derivative was determined by an immunoassay from Optora Chemicals. PGEL Prostigizatin D. (PGD2), and Beprostone, PGE, and 8.

Western Bits Cells were presented with tocopherold for as least to absurbed above, Optimal protein induction of COX-3 and induable mirrie oxide sputhase (NOS) in RAW264.1 cells seed observed at 8 and 14 h, respectively, after LPS treatment. Harvasted cause by the protein in Harvasted cause of the protein in Heapt by the protein in Heapt butter with protein by societation in Heapt butter with protein as well among the protein of the protein concernations. SDS/PAGEseasperformed management (Organ Los COX-2 and 1/5% gelf (Box Rad) for INOX. Reabted protein were managed by either with the protein were managed by either with the protein were frast or the bitter hollogy but in compagued to home addition in the probed by primary authodics and secondary authody (State Protein). Which Life Secons Produch) Proteins were exampled to a Modak Riam y using a NUSA X. DNAT processor (Kodal), and that was a Nush X. DNAT processor (Kodal). The Butter DNAT processor (Kodal) Proteins and the Nush X. DNAT processor (Kodal). The Read of the Nush X. DNAT processor (Kodal).

tocopherits for 8-14 h and then stimulated with LPS (as 8 b. or Toral RNA was purified by using RNCary Miniprep Lit (Gingan, Charworth, CA), and 3 leg (LNA was motioned to a grave part and or authorized outs a positively changed oplan menthane (Roche Moberular Biochteniauls). Hybridization probes for COX-2 and Futton were spullerized by wing fairt cDNA as templates for PCR, and labeled with digangement 14 city (Roche Moberular Biochteniauls). The mRNAs on the mem-Korthen Blot. RAW164.7 macrophages were prefreated with

Inter were detected by chantilumines and this (Roche Molecula) Biochemicals) and quantified by purise quart. Strictical Analysis. The impaired Student's t rest was used to the statistical analysis. All results are expressed as means z SDs.

LPS. Supplementation with 7T led to a done dependent reduc-tion of PGH, synthesis (Fig. 14), with an apparent ICm of 73 = 24 to contrast to 47, at with concentrations of thest than 10 pM had fulle effect on PGE, production, whereas 10 pM PCBHC thorsed = 10% unbilition, A19 pM, yT and PCBHC in yn—978 and 40% reduction of PGE, synthesis, respec-tively, whereas of thorsed only = 150% tabbition. Differential Mecro et al., 11, and FICING on FGE, Gamertian In RAWJELT Cells Stheuland with 10% and Epithalial Cells Treated with R-16. A manked timmase of PGE, generation was observed to, RAWZELT macomplage when cells were treated with A.1 µg/ml

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to evaluate the anti-failumment effect of conversibles and influences of days in closest cell (2). In LL piercale A450 cells both y's and "CEIIC cubbiced date-days advantage of PCE; release, with LCs of 4 ± 1 and sad 10 ± 1 pink respectively (Fig. LB). On the other hand, of add on those any effect own at a concension of the physical physical observed in the presence of exagenciar AA of T was also observed in the presence of exagenciar AA. (Fig. 1C).

premoubated with yT or of for \$1.4 to before LPS or 11.19 to remoup the detail of the fore the left of the fore LPS or 11.19 to remove the detail of the foreign that the cells to remove free yT before the treatment only alguly diminished the inhabitery perceive, it concent, perceived to a lost or required for the optimal inhabitery nearest, in the second to the optimal inhabiter of the second to the difference only be early placed by designed with the difference only be early placed by designed either in a susceived with the optimal for the yT molecule in contrast to the more bythopologicity of the yT molecule in contrast to the more bythopologicy of the yT molecule in contrast to the more bythopology. for yf was affected by the serum amount to the medium during LPS frequent. PRB (frambfin) was — 10–20% to not potently libbilitied by yf when cells were frequed with LPS in the frequence containing 0.5% FBS compared with those in 10% FBS (data not Maximum inhibition of PCE, was achieved when cells were

The difference between 1T and of I in inhibiting POE, ynthere is likely not crusted by their differential cultular interporation. Under our experimental conditions, holds decoplemits
were rimitally incoled by mescophieges and the apparton. The
companion was -30% higher than of its burnars epith oells (see Materials and Methods).

effect on other Aradidaars derkobetica. Consistent with a previous report (28), we found that baider DOBs. I.P.S. trented PCDs. VEAT managed speciated industrial amounts of PCDs. (Tible I), in vivo supplementation of YT little III to recharge the tabletics of PCDs, tymbests, although the tabletics of PCEs. was lightly but significatly dots poten. This riggets that 'I'l analy alloca the common step in the spathetis of different profusiglandine Stables to the case with PGE, all bad no effect on PGD, release 11 to Jul.

Another AA metabolite, 8-tsuproutnes, but been recognized.

by with yt of all saused agmiticus induction of legiparatine formation, and yt is significantly more potest than at [[Table I], ~60 and 25% reduction by 10 mM yt nod at, respectively. as a servicine and specific maskes of lipsed peroxidation under oridative stress (27). LPS strengthon of outrophage led to a musted increase in the refease of Biroproclane. SupplementaPHAS | OF ENGLIQUE | WLST | PAST | 1 1945

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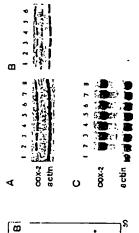


Fig. 1. Effects on COT-1 as pression. (A) Wenterin Bots of RAVIZEd, I married pression content and the content of the Cott. (C) to give in the content and the content of it is local, the major and also and the content of it is local, the major of the content of

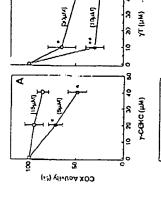
cells was extended to 8 or 24 h, which allowed substantial cellular meorwhen the incubation of 7T with IL-18-pretreated

We ad (3) reported that in who supplementation of at Tory T to old miss relu caused display inhibition of POB, symbols. The disrepancy with the present study is likely due to the different systems used. It is known that compared with symp cell, cells from old animals have much lower anisondant capacity and significantly higher levels of third hydroperoxide (3), 14) that ponition, we observed an invibitory effect (Fig. 1).
The effect of A Cannot be ambured to its authoridate activity
because both YT and AT exhibited soular ability to shibit
DCFH oridation (Fig. 4), whereas of Boned a march kes porcet
effect on PCEs, symbass. When this paper was in preparation. lead to an embanced COX serivity (13). Connequently, o.T. a powerful authoritates that decreases lipid hydroperatorite in old notonia, reduced PGE, referes to old cetà (33), Constuently, no inhibitory effect was observed when of was supplemented in

young rate (15, 26).
yThat a normalistrated 5-position, making it a bener nucleo. pale for hypping electropolies and a pronger indibitor of percognitude difficient formation (17) as compared with ut. Although perasyminite has been suggested us a perastide source for COX-2 activity (26), in the current system, peroxy trite is not likely to be a major mediator because POE, synth

or inhibitory polency of 17 was our aftered by NO (Table. The physological Nurre of pervaide is thus unknown, A comprobenire understanding of the action of yT was also impeded by our unsuccestful aftempt to real its includings effect with the purified energie (data not shown). Nowever, our results from idnet cells inggest that yT and yCEHC may serve as weak compositive inhibitors of COX-2 because their inhibitory potency was deribathed wide an intresse in AA concentration (Fig. COX.2 or inhibition of third peroxidation as a nucleaphile (17), It remains to be determined whether the inhibitory activity 7T is caused by its competing with AA at the binding pite of

Numerour mudies demonatrate that nonstrenidal anti-lathamatory drugs exert their therepeutic effects by inhibiting COX accivity (33). Day potteons estimated in intac cells, compared with using porified entropes, none accurately reflect their harding as COX tabbifors is nino (24, 38). In the present thinly, y?! served as a COX tabbitor in strapt cells but non with diod.



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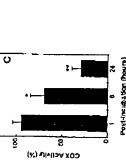
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Tocopherois (µM)

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Hg. I. Onterratal cried of al. 11, and pCRK on PCB pertition in namedy by and schinds oils (all Existing Tests over protochated with all 11, or schools and the state of the schools of th

Coty set advocad Sade with ASS with worn promated with the Process of the Part of the Part

inhibitory effect with an ICs, of =30 mM in both cells. In contrast, of 21 50 mM goaly algaby reduced (25%) PGPs, formation in markey have, but had so inhibitory effect in humo-epithetial cells. We also observed an inhibitory effect of yT and yCEHC on FGE, geograpion in the primary busine lang

1C) demonstrates that the effect is not caused by a decrease in physophological As artifly of instrate availability. ACERIC, but out Y. Caused to innodule decline in Fifty, telescaped to the exposure to the IL 14 peretected AS49 cells followed by addition for exposure and ACERIC, whereas in probably that to a more myst exposure of expectation ACERIC, whereas the cellular incorporation of YI mushly rakes hown (23). Consistent with this hypothesis, The inhibitory effect of yT stons from in direct jahlbition of metabolite pouces anti-inflummatory properties.

> 3.5 ± 1.7°1 20 ± 25 °° 20 ± 0.6°* والمار 10 PT

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78 ± 0.9 248 ± 15 5.5 ± 0.4* De 100 100

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8-Acprovene

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Inmonethyl-L-argidue (L-NNMA), a ipecific inhibitor of INOS, to test whether NO has any effect on yT-centred PCIE, inhibitiso. Under our repentingual windstidins, the blocking of NO formation by LaM L-NKMA did not affect PCIE, synthesia

LPS neatment led to a marked increase in iNOS expression in macmphases. Both a T and yT inhibited DNOS expression at 10 or the labilitiony potenty of TT (Table 1), indicating that PRE, formation is probably on acdisted by NO.

tots I wan preson

Control

AA metabolite

Post-incubation (hours)

õ

phi (Tebe 2), although at at the concentration did significantly suppress nitrite releases.

*febroon of COX activity by posteroubsticm with yT and p-CLMC in

his been used to evaluate the total generation of reactive corgan poechs including superorde, hydrogen peroxie, tod peroxyninite (29). In RAWSA? Tracryphyses, LPS treatment caused a

inhibition of Bustine Orygen intermediates. Oxidation of DCFH

inhibitory threat of 13 and years. On CBA2 Acothy is 11-19-14. COX-2 continues and years of y

Algundaet tornuse in fluorescel intensity compared with con-oritis (P < 0.05, Fig. 4), indicating a marked sententing of reviews orienters species. Other baries with all 191, and PCEHCled to the similar inhabition of DCFH oridation, suggesting than they

and B), suggesting that y T and y CEHC might compete with AA I the sective rife of COX-2. COX-2 protein levrib wree not affected by the prathiculation with y7 (data not aboun).

possess temular antioxidant activity.

Discussion

thethe on (03.1 tepression. A highfleant instease in COX.2 be repression was observed in LPS, heated manophages on IL-16. C standard organizated organization of IL-16. So table, and the rempared with untressed or the 16. Up to in 90 th 17. The The CELIC did not shown significant imbiblings of COX.2 prostic arpsociate (Fig. 3.4 and 8). Consistently, a Northern blots showed that the induction of COX.2 bushNA was not effected by in vitro supplementation of COX.2 bushNA was not effected by in vitro supplementation of coxpletely (Fig. 3.5C).

11hcts on Nicrie Ledwase and Dros Expression, Nicrie accumulation was movel organic or evaluate the potential effect of toxopherols on NOs formation in LPS-braised maximplages. At 10 ani, yt 188 http (nhibit) (=188, P < 0.0.0) mitrie referse (Table 2),

whereis on ther of nor pathe is solificially reflect in nearwied lo four iadepeaden experiments (data not thom). Because affice ande has been shown to postentiale PGE; synthesis in LPS-meated machiphages (28), we used Advisormethyl-larginiae (LNNMA), a specific inhibitor of

C) docay geosso-catalyred prostegandio synthesis is one of the inportain early events thring inflammation. As a key inflammatory mediator, PGB, is known to stimulate cytokine greetfibroblasts when treated with U.18 (unpublished observation). These results demonstrate that yT and its major water-soluble

abon (30), cause reactifusion, and mediate fever and pain (31). A major finding of our carrent study to that "T is physiologically perfected constructation tablistic PCB, generation to two lodes printed call thousand PCBs, synthasis in a LPS, and, and to Eles synthasis in a LPS, and, and to Eles printed in the LPS and, and to Elefter the major metabolite of YT, YCEHC, etabliste a similar thin. The major metabolite of YT, YCEHC, etablised a similar

Table 1. Effects of all and y7 on the generation of different AA metaborities in LPS-treated marrophages

Amount released, ng/10º cells

cyckoarygenese activity in inteat cells as above by its mithition (by 60-80%) of PGE, synthesis and its lack of significant effect on COX.2 expressions at concentrations of 10-50 µMs. That the lahibition was observed in the presence of exogenous AA (Fig.

I to Grait

RAWRA 7 cells were preforduled with whole (correct), of, or yi for left is and orande with 01, aginal LPS for 14 h. AA meri before released in the modern were measured +, P < 0.05 and +, P < 0.01, control in +jf or a? Peptermenthion.

Table 2. Fffict of toxopherals on nitrite accumulation and IKOS expression

		illing assessment of the	* Vone who	Ē	69 ± 12.	76 ± 10*	
	1 49/10 CHIS	CAYMINA (1 min)		8.4 ± 1.D	1.7 ± 0.8 • •	0/	e e
	2	Control			0.0	2011	
Nicrity seleane, MA	1	L'AIMAGA (8 MAR)	2.1 = 0.7	70:27	9	ē	
	logod)		74.0 ± 3.1	194 = 1.6	27.1 = 1.4	3.1.2.5	
	Ireatinem	15	.r. (111 6.0	20001	Dem oil is	La Company	

RAVY 64, ENT was palinubandarible dis materitica los places for laband entre side with a spatial LPI in the present and state (control of simply labels and services). As seed control of simply labels and services are controlled and services are controlled and services are controlled and services. The services are controlled and services are controlled and services are controlled and services are controlled and services. The controlled and services are controlled and services are controlled and services are controlled and services. The controlled and services are controlled and services are controlled and services are controlled and services.

the puritied entyme, similarly to codium adiculate, which inhibited collishes per systems with an ICo of 23 key but did not show shaking with the priced OOX 7 (24). Altimugh further in the current finding is consistent with our retart observable with the retart of the retard of the Gently incompranted in the call. Further experiments are needed to understand the mexican or iNOS inhibition.

Amparable to the yT ICss values (4-10 p.hf) estimated here in YT concentrations in burness plasmes way from 1 to 6-7 mM (42), detending on the animot of dietary intake. Draw levels are

17.5 based 2000 38 8 88 _ UMU (IDMICIA) #3nesemoni3

Rg. 4. Toda navive dagen upolo formeton by DCMs awy, in general portra. 5x 1x 6x 6x pa well-june inducerol (or at least 10 tol) in enhancem (or at least 10 tol) in enhancem (or at least 10 tol) in an analysis of told away to the order of told away then be reted with 6 up final [b] to 1b, washed have a with teach blance suffice and finally fundersed with 40 jud DCMs (or a h or a overnight, v, P c anscomptive to opherol upplementation.

This best few die universe physiospecially referrant. Although, 17 have best few die 14 + 5 litters and or 4 hands in die ließ (5), there have been rearly conflicted an adjone riesues (4), 44). Burnan the best north conflicted an adjone riesues (4), 44). Burnan die (4) that we reachly reported that 17 constituted 10-50% of 17 constituted 10-50% a hytrophilic product povessing the state bulballand to +CRHC (44), and therete physyl chain with a carbonylate stall +CRHC is possessing the state echoemono | Indeed to the coopiage at a notiverse state of the stall +CRHC is plasma econcentration in -SO-185 ab (47). Its freet gast be high yT distale. We show that +CRHC is a COX.2 inchibitor with an important ICs of 10 tub. which of the state minet cells, and this seem physologically releases. Although if

duding human colon cancel, leve been reported to cantain co haced COX-2 expression and PGEs (45). PGFs but been store to parmote pradiceution in cantain cancer cells and constructed and inflaturatory drugs can tabbit the growth of cantinens cells Our current factings that if and in meakelite +CEHC powers and informationy properties may have important physiological implications. First various actional and turnan hance cosen, to coloreral racer to provide a your experse. In visione run to numerate designated observations are recorded and information drugs (44–71). Causer and (16) as bon in CH 1011 A instance of the preventing compared random bits effect to rai fully understood, we propose that the ability of your childless of the factorial recorded are proposed that the ability of your recorded to this effect. and appress acgoseness (48). Consequently, sowral population-based ancles bare detected a 40-50% deprates in relibre rule for

to addition, inflammation plays a key role in the Initiation and development of atheroxelerosis and augmented expression of COX.2 has been found in human atheroasteropic lossoss but not io normal uterics (12). The auti-influentiatory properties of yT may be important to preventing entitional disease. Two clinical indicate opened that serial relate of yT, but not all, were reduced to coronary boan dist are patiens (11, 13), to one of the epidemiological studies, Kushi et al. (33) found that the tutale of vitamin E from diers, which comuse society 71, but on supplement, which conden prodominately of a 7, was stressely

other hand, studies by Stampler at al. (9), though on directly comparable, dad not observe the same effect. The recent somal studies by Safoern ed. (14) indicate that "I supplementation in Sprague-Dawley tast, compared with oT, showed more plateful supplementation of low density imprinting undation, plateful significant on flow density imprinting undation, to subatary, our current strong-our current strong-our current strong-over current stro

In suitamery, our current study demonstrates that 7T and its major mestabolis, but not of thinhold COX accivity and thus possess such inclinations against 70 to 14 set combined with the cited browns and salarus treatles tregget that 7T may be impor-

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Ne thark Dr. L. J. Marrett, S. Christeo, M. K. Shigranga, S. L. Haren, and K. Satzer, for evillate retiren, of this instruction. This work was supported by a Participant of Heart Associated by a Participant of Heart Associated Western Atthicts Grant 18-24 (Q.)., the Wheeler Mand for the Beological Science at the University of Calibratia Retirety, and the National Lording of Environity of Calibratia Retirety, and Exollege (UNIA.).

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